C.F.R. §1.821(e). As required by 37 C.F.R. §1.825, Applicant's Agent hereby states that the content of the Substitute "Sequence Listing" in paper form and the computer readable form of the Substitute "Sequence Listing" are the same and, as required by 37 C.F.R. §1.825, also states that the submission includes no new matter.

Please amend the application as follows:

In the Specification

Please replace the "Sequence Listing" filed on December 2, 1996 (pages 118 through 146) with the attached Substitute "Sequence Listing" (sheets 1/25 through 25/25) comprising SEQ ID NOS: 1-67.

Replace the paragraph at page 7, lines 3 through 7 with the following paragraph:

Figure 7 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand, SEQ ID NO:11; non-coding strand, SEQ ID NO:64) encoding the mouse Act-1 light chain variable region joined to the mouse Act-1 light chain signal peptide sequence, and the deduced amino acid sequence of the Act-1 light chain variable region joined to the mouse Act-1 light chain signal peptide sequence (SEQ ID NO:12).

Replace the paragraph at page 7, lines 8 through 11 with the following paragraph:

Figure 8 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand, SEQ ID NO: 13; non-coding strand, SEQ ID NO:65) encoding the mature human GM607'CL antibody kappa light chain variable region, and the deduced amino acid sequence of the mature human GM607'CL antibody kappa light chain variable region (SEQ ID NO:8).

Replace the paragraph at page 7, lines 12 through 23 with the following paragraph:

or K

Figure 9 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand SEQ ID NO:14; non-coding strand, SEQ ID NO: 66) encoding the mouse Act-1 antibody heavy chain and signal peptide, and the deduced amino acid sequence of the mouse Act-

1 antibody heavy chain variable region and heavy chain signal peptide (SEQ ID NO:15). The nucleotide sequence of the variable region is joined to a nucleotide sequence which encodes a deduced mouse Act-1 heavy chain signal peptide sequence, to yield a composite sequence. (The identity of the primer which amplified the heavy chain region was deduced from the degenerate sequence, and an amino acid sequence for the signal peptide was derived from the primer, downsteam sequence and sequences of other signal peptides. The signal peptide shown may not be identical to that of the Act-1 hybridoma.)

Guil

Replace the paragraph at page 7, lines 24 through 31 with the following paragraph:

6,5

Figure 10 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand SEQ ID NO:16; non-coding strand, SEQ ID NO: 67) encoding the human 21/28'CL antibody heavy chain and signal peptide, and the deduced amino acid sequence of the human 21/28'CL antibody heavy chain variable region and heavy chain signal peptide (SEQ ID NO:17). The nucleotide sequence encoding the variable region is joined to a nucleotide sequence which encodes a signal peptide sequence derived from the V_H of human antibody HG3'CL (Rechavi, G., et al., Proc. Natl. Acad. Sci., USA 80:855-859 (1983)), to yield a composite sequence.

Please replace Table 3 at pages 53 through 63, with the following Table 3 presented on pages 4-14 of this Amendment:

MAY 2 3 2003

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions. Table 3.

Comment		Canonical AA for L1 loop (a1).		Buried between L1 and L2.	V=9/245, M=202/245 in	mouse k-II. M=42/45, V not seen in	human K-II. If binding is poor, consider	changing inis to vat in second version.			Distal to binding site	(53). T=164/245 in mouse k-	T=10/37, S=27/37 in	numan k-11.		
Surface or Buried		buried	r	buried							surface					
Act-1 or RH V _K (SEQ ID NO:52)	, α	Λ	Λ	M					Т	8	SI			d		T
GM 607CL (SEQ ID NO:8)	Q	I	Λ	M					Т	0	S			ď	•	T
Human K-II (SEQ ID NO:51)	D*	1*	۸*	M					Т*	٥*	S			*d		L*
Mouse r-II (SEQ ID NO:50)	D*	Λ	۸	M					Т*	٥*	L			d d		Ţ
Mouse Act-1 (SEQ ID NO:7)	D	^	۸	>					Т	Q	Ţ			ď		IJ
FR or CDR	FRI											_				
#	1	2	3	4					2	9	7					6
Kabat	1	2	3	4					2	9	7			80		6

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse ĸ-II (SEQ ID NO:50)	Human ĸ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V _K (SEQ ID NO:52)	Surface or Buried	Comment
	01		S	S	*S	S	S		
	=		L	ı	*1	L	L		
	12		Ь	<u>a</u>	ď	Р	P .		
	13		^	*>	*>	^	۸		
	41		<i>ω</i>	v	‡.	-	H	surface	Distal to BS. S=151/248 in mouse k-II. T alone (30/30) seen in human k-III.
	15		Ĺ	٦	۵	۵.	⊡	surface	Distal to BS. F=9/253 in mouse k-II, F not seen in human k-II. P=29/31 in human k-II.
	16		5	*5	*5	U	Ŋ		
	17		Ω	D	ш	ய	ш	surface	Distal to BS. E=18/30, D not seen in human k-II.
	18		0	Ø	*	<u>a</u>	ФI	surface	Distal to BS and on a turn. P alone (31/31) seen in human k-II.

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont').

				Т	$\neg \neg$						
Comment	Pointing into core, but standard mouse to human change. V=66/253, A=187/253 in mouse k-II. A alone (30/30) seen in human k-II.						Canonical AA for L1 loop.	Canonical AA for L1 loop.	Canonical AA for L1 loop.	Canonical AA for LI loop.	Canonical AA for L1 loop.
Surface or Buried	buried										
Act-1 or RH V _K (SEQ ID NO:52)	⋖ I	S	I	S	C	R	S	S	ð	S	L
GM 607CL (SEQ D NO:8)	V V	S	I	S	C	R	S	S	0	S	1
Human k-II (SEQ ID NO:51)	**	*S	*_	*S	. 2	R	**	*	0	S	L*
Mouse K-II (SEQ ID NO:50)	4	*S	I*	*%	ť	R	*»	**		w	1
Mouse Act-1 (SEQ ID NO:7)	>	S	I	S	C	R	S	S	0	S	Г
FR or					FR1	CDR1					
#	10	20	21	22	23	24	25	26	27	78	29
Kabat	61	20	21	22	23	24	25	26	27	27A	27B

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont')

	1					T			T		-
Comment	Canonical AA for L1 loop.	Canonical AA for L1 loop.	Canonical AA for L1 loop.		Canonical AA for L1 loop.	Canonical SS for L1 loop.	Packing AA. Unusual (117/1365). A, H and N most commonly seen here.				
Surface or Buried										-	
Act-1 or RH V _K (SEQ ID NO:52)	•	Ж	·		¥	ŭ	Z	T	Y	L	ν.
CL CL (SEO ID NO:8)		н	S	1	z	Ð	*	z ·	*	ני	Q
Human k-II (SEQ ID NO:51)		н	N	×	۵	D	Z	z	*.	*1	z
Mouse K-II (SEQ ID NO:50)	>	Н	S.	1	Z	.	z	Т*	* \	r.	ш
Mouse Act-1 (SEQ ID NO:7)	4	×	S	1	*	G	z	Ţ	¥	Г	W
FR or											CDR1
#	30	31	32		33	34	35	36	37	38	39
Kabat	27C	27D	27E	27F	28	29	30	31	32	33	34

-7-

Table 3.	¥	lignmen	t of amino acid	Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont.)	in the design of	reshaped human	mAb Act-1 V _L	regions (CC	int)	
Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse k-II (SEQ ID NO:50)	Human ĸ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V _K (SEQ ID NO:52)	Surface or Buried	Comment	
35	40	FR2	W	W*	M*	W	W			
36	41		¥	Y	Y	Y	Y		Packing AA. Most common AA.	
37	42		Т	r*	ı	L	L			
38	43		н	*>	8	8	a	buried	Packing AA. H is unusual (31/1312). Q is most common	
									AA (1158/1312). H=6/225,	
									Q=219/225 in mouse k-II. Q=15/17, H not seen in human k-II.	
39	44		К	×	К	K	К			
40	45		Ф	*	Ъ	P	P			
41	46		Ŋ	" "	G*	G	. D			
42	47		ð	۰۵*	Q	0	0		·	
43	48		S	**	S	S	S			
44	49		Ь	P*	P*	ď	С		Packing AA. Most common AA.	
45	20		0	м	0	0	ø			
										٦.

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont') Table 3.

	nt	n AA.		A for		A for	A for	A for								• }	
	Comment	Packing AA. Most common AA.		Canonical AA for L2 loop.		Canonical AA for L2 loop.	Canonical AA for L2 loop.	Canonical AA for L2 loop.									
	Surface or Buried					. •											
	Act-1 or RH V _K (SEQ ID NO:52)	L	Т	. 1	Y	S	I	S	Z	R	Ħ	S	G	Λ	Ф	D	R
	GM 607CL (SEQ ID NO:8)	T	L	I	Y	L	Ð	S	Z	R	A	S	Ð	>	A.	D	R
	Human k-II (SEQ ID NO:51)	L	L	*1	Å *	T	>	**	z	R*	¥	S*	* 9	*^	P*	Ω	×
	Mouse k-II (SEQ ID NO:50)	L	*1	*	Y	K	>	*	Z	R	ГT	S*	*5	*^	Ь	D *	κ *
	Mouse Act-1 (SEQ ID NO:7)	I	T	I	Y	Ŋ	I	S	z	R	Ħ	S	ß	>	ď	Ω	R
,	FR or CDR				FR2	CDR2						CDR2	FR3				
	#	51	52	53	54	55	99	57	58	59	09	19	62	63	49	65	99
	Kabat	46	47	48	49	20	51	52	53	54	55	Se	57	28	59	09	61

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont') Table 3.

Kabat	# .	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse ĸ-II (SEQ ID NO:50)	Human ĸ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V _K (SEQ ID NO:52)	Surface or Buried	Comment
62	29		ш	# #	* *	H	F		
63	89		S	Ø	*%	S	S		
49	69		D	*5	Ð	Ð	g		Canonical AA for L2 loop.
65	70		S	**	S*	S	S		
99	71		g	.	G*	G	Ċ		
<i>L</i> 9	72		S	*%	S	S	S		
89	73		ŋ		Ð	Ð	Ð		
69	74		Т	*L	T*	Т	Т		
70	75		D	D	Q	. а	D		
71	76		Ŧ	* [L	* [14	H	F		Canonical AA for L1 loop.
72	77		T	T *	*L	Т	Т		
73	78		L	T*	Ľ*	1	Г		
74	79		×		K	Ж	Ж		
7.5	08		I	*1	I*	I	I		
92	81		S	S	S	S	S		
	-								

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont') Table 3.

Comment	Distal to BS. T=6/221, R=211/221 in mouse k-II. R=11/12, T not seen in human k-II.	Pointing into core, but standard mouse to human change. I=6/213, V=195/213 in mouse k-II. V alone (12/12) seen in human k-II.	Distal to BS. K=20/215, E=191/215 in mouse κ-II. E=9/12, K not seen in human κ-II.	Distal to BS. P=6/183, A=175/183 in mouse k-II. P=1/12, A=11/12 in human k-II.
Surface or Buried	surface	buried	surface	surface
Act-1 or RH V _K (SEQ ID NO:52)	씸	>1	மி	∀
GM 607CL (SEQ ID NO:8)	<u>م</u>	> .	Щ	∢.
Human k-II (SEQ ID NO:51)	~	*^	山	∢ .
Mouse K-II (SEQ ID NO:50)	**	>	ш	*V
Mouse Act-1 (SEQ ID NO:7)	T	I	×	Q
FR or CDR				
#.	83	83	84	85
Kabat	77	78		08

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont') Table 3.

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-Π (SEQ ID NO:50)	Human ĸ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V _K (SEQ ID NO:52)	Surface or Buried	Comment
81	98		щ	E*	Е	Е	В		
82	87		D	D*	D	D	D		
83	88		r	T	*^	۸	N	half buried	Dital to BS. V alone (12/12) seen in human k-II.
84	68		Ð	*5	G *	G	G		
85	06		×	>	*^	>	>I	half buried	Distal to BS. M=6/212, V=196/212 in mouse k-II. V alone (12/12) seen in human k-II.
98	91		7	<u>۲</u> *	Å *	Y	¥		
87	92		7	X	Λ *	¥	Y		Packing AA. Most common AA.
88	93	FR3	S	*	* 2	С	C		
68	94	CDR3	1	(L)	*W	M	T.		Packing AA. L is unusual (93/1238). Q is most common AA (654/1238).
06	95		ð	*>	ð	0	ð		Canonical AA for L3 loop.

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont')

Kabat	#	FR or	Mouse Act-1 (SEQ ID NO:7)	Mouse k-II (SEQ ID NO:50)	Human k-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V _K (SEQ ID NO:52)	Surface or Buried	Comment
91	96		O	Ö	A	V V	9		Canonical for L3/Packing AA. 3 rd most common AA.
92	97		T	L	1	T	T		Canonical AA for L3 loop.
93	86		Н	Н	0	8	н		Canonical AA for L3 loop.
94	66		ø	>	· ×	T	0		Canonical AA for L3 loop.
95	100		ď	p *	Р	Ъ	P		Canonical AA for L3 loop.
95A				Ъ	R*		•		
95B				ı	1		ı		
95C			1	ı	ı		ı		
95D			•	1	ı		1		
95E			1				ŧ		
95F			1	1	ı		1		
96	101	<u>-</u>	Y	Y	×	0	X		Packing AA. 2 nd most common AA.
97	102	CDR3	Т	T*	T*	T	Т		Canonical for L3.

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_L regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse k-II (SEQ ID NO:50)	Human ĸ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V _K (SEQ ID NO:52)	Surface or Buried	Comment
86	103	FR4	įτ,	*	*	Ħ	Ŧ		Packing AA. Most common AA.
66	104		9	*5	* 5	Ð	Ð		
100	105		Ö	Ð	8	Ø	δ	half buried	Distal to BS. Q=12/13, G=1/12 in human k-II.
101	106		g	*5	G *	Ð	Ð		
102	107		T	*_	Т*	Т	T		
103	108	_=	×	K*	X	Я	К		
104	109		ח	*1	>	>	>	half buried	Distal to BS. L=5/14, V=9/14 in human k-II.
105	110		ш	E *	E	a	ш		
106	111		I	I	*I	. 1	I		
106A			ı	,	1	1			
107	112	FR4	K	K*	K	. Ж	K		

Please replace Table 4 at pages 65 through 77, with the following Table 4 presented on pages 16-28 of this Amendment.

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions.

Kabat	* ,	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ ID NO:55)	Surface or Buried	Comment
1	-	FR1	Q	ð	ð	ð	8		
2	2		^	*^	۸	Λ	>	·	
3	3		0	*0	*0	δ	0		
4	4		L	L*	L*	Г	T		
S	Ŋ		~ ~	ò	۸	Λ	>I	surface	Distal to binding site (BS). Q=135/143 in mouse IIB.
									V=49/53, Q=1/53 in human I.
9	9		0	0	ð	0	0		
7	7	— —	ď		ν*.	S	ଠା	half buried	Distal to BS. P=102/150 in mouse IIB. P not seen in human I.
&	∞		Ö	. 9	. 9	Ð	Ö		
6	6		Ą	А	А	Ą	Ą		
10	01		ш	Э	3	Е	E		
11	=		ı	r.*	>	Λ	>	surface	Distal to BS. V=50/54, L= 4/54 in human I

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont')

Comment	Pointing into core, but standrd mouse to human change. K=41/55, V=3/55 in human I.				Distal to BS. T=12/139, A=117/139 in mouse IIB. T=1/52, A=23/52 in human I.			
Surface or Buried	buried				surface			
Act-1 RH V _H (SEQ ID NO:55)	⊻ I .	¥	ه	O	∀ا	v	>	×
human donor 21/28CL (SEQ ID NO:10)	×	Ж	a.	9	<	S	^	×
Human I (SEQ ID NO:54)	×	K*	*d.	*5	∢	*	>	Ж
Mouse IIB (SEQ ID NO:53)	* >	Ж	* .	* 5	¥	*0	*^	К
Mouse Act-1 (SEQ ID NO:9)	Α	м	ď	Ð	Т	S	Λ	К
FR or CDR								
#	12	13	14	15	16	17	18	19
Kabat	12	13	14		91	17	18	19

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont')

Comment	Pointing into core, but standard mouse to human change. L=138/179 in mouse IIB. V=36/52, L=1/52 in human I.				Canonical AA for H1 loop (A1). G not seen in mouse IIB. G=12/51, A=34/51 in human I.	Pointing away from BS and so does not appear to bind antigen (Ag). Y=1/185 in mouse IIB. S=48/50, Y not seen in human I.
Surface or Buried	buried				buried	surface
Act-1 RH V _H (SEQ ID NO:55)	>	S	C	ж	v	
human donor 21/28CL (SEQ ID NO:10)	>	S	C	Ж	∢	w
Human I (SEQ ID NO:54)	>	S	*	K	¥	* %
Mouse IIB (SEQ ID NO:53)	1	%*	* 2	K*	A*	*x
Mouse Act-1 (SEQ ID NO:9)	7	S	С	K	D	Y
FR or						
#	20	21	22	23	24	25
Kabat	20	21	22	23	24	25

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ ID NO:55)	Surface or Buried	Comment
26	26		Ð	*9	G*	Ð	9		Canonical AA for H1 loop.
27	27		Y	. X	Y	Ā	Å		Canonical AA for H1 loop.
28	28		T	L	Т	Т	Т		Canonical AA for H1 loop.
29	29		ţ r ,	*	F*	F	F		Canonical AA for H1 loop.
30	30	FR1	H	T .	Т	Ţ	Т		Canonical AA for H1 loop.
31	31	CDR1	S	S	S	S	S		Canonical AA for H1 loop.
32	32		Υ	Y	Y	Y	X		Canonical AA for H1 loop.
33	33		w	W	A	A	M		
34	34		M	M	I	М	М		Canonical AA for H1 loop.
35	35		Н	Н	S	Н	Н		Packing AA. Most common AA.
35A				ı	-	•			
35B		CDR1	1	ı	•	4			
36	36	FR2	W	W*	W*	W	W		

ıt')	Comment	Packing AA. Most common AA.	Pointing into core, but standard mouse to human change. K=177/188 in mouse IIB. R=48/49, K not seen in human I. However, Lys maybe packing H2 loop, therefore consider changing in second version, in conjunction with A40R, if binding poor.	Packing AA. Most common AA.
gions (Con	Surface or Buried		buried	
Ab Act-1 V _H re	Act-1 RH V _H (SEQ ID NO:55)	^	ଧ	0
Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont')	human donor 21/28CL (SEQ ID NO:10)		Na diagram and a second a second and a second a second and a second a	Ò
ed in the design	Human I (SEQ ID NO:54)	>	R*	*0
id sequences use	Mouse IIB (SEQ ID NO:53)	>	₩	0
ent of amino ac	Mouse Act-1 (SEQ ID NO:9)	>	⊻	Ø
Alignm	FR or CDR			
	#	37	88	39
Table 4.	Kabat	37	38	39

nt') Comment		Pointing into core, but standard mouse to human change. R=160/177 in mouse IIB. A=37/49, R=0/49 in human I. However, Arg maybe packing H2 loop, therefore consider changing in second version, in conjunction with A38K, if binding poor.				Pointing into core, but standard mouse to human change. G=43/48, R=5/48 in human I.
Surface	Buried	buried .				buried
AACt-1 V _H re Act-1 RH V _H (SEQ ID NO:55)	·	∀ I	Ь	Ð	0	ద
Sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont') Mouse IIB Human I human donor 21/28CL Act-1 RH V _H Surface EQ ID NO:53) (SEQ ID NO:54) (SEQ ID NO:56) or			Р	Ð	0	æ
ed in the design Human I (SEQ ID NO:54)		Ą	P	Ð	0	Ð
cid sequences us Mouse IIB (SEQ ID NO:53)		x	Ъ	Ö	Ò	U
Alignment of amino acid FR or Mouse Act-1 CDR (SEQ ID NO:9) (S		x	P .	G	Ò	U
Alignm FR or CDR						
#		04	41	42	43	44
Table 4. Kabat		0	41	42	43	44

46 46	FR2 G CDR2 E CDR2 E CDR2 E CDR2 E CDR3 E CDR4 C CDR5 C CDR6 C CDR6 C CDR7 C CDR7 C CDR6 C CDR7 C C C CDR7 C C C C C C C C C C C C C C C C C C C	Andree IIB (SEQ ID NO:53) L* E* G* G* D D D	Human I (SEQ ID NO:54) L* M M M I N N Y	W W W W W W W W W W W W W W W W A W	Act-1 RH V _H (SEQ ID NO:55) L W G G I D P	buried buried	Packing AA. Most common AA. Most common AA. Ile Underneath and supporting H2 loop (a2). Met=41/48 in human I. Canonical AA for H2 loop.
		. , ;		, 0	U		Canonical AA for
53 54	S	Z	g	Ŋ	S		Canonical AA for

able 4.		Alignm	ent of amino ac	id sequences use	ed in the design	Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont')	Ab Act-1 V _H reg	gions (Con	t')
Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ ID NO:55)	Surface or Buried	Comment
54	55		E	S	Z	N	E		Canonical AA for H2 loop.
55	99		·	9	G	9	· S		Canonical AA for H2 loop.
56	22		Z	9	D	N	N		
57	88		T	Т	T	I	T		
58	65		Z	Z	Z	.: У	Z		
59	09		Ā	Ā	Ā	Ā	Ā		-2
09	61		N	N.	Y	S	N		
61	62		Q	ਜ਼	ð	ð	ð		
62	63		K	K*	K	К	Ж		
63	64		··	*T	Ā	Ŧ	Ŧ		
64	99		K	K	ð	ð	К		
65	99	CDR2	G	S	G	G	9		
99	<i>L</i> 9	FR3	м	K*	M.	M.	씸	surface	Distal to BS. R=39/49, K not seen in human I.
67	89		A	A*	Λ	Λ	>	half buried	Distal to BS. V=45/48, A not seen in human I.
89	69		Т	T*	Т	L	Т		

-23-

		- I							I	į.
ıt')	Comment	Leu Underneath and supporting H2 loop (a3). Ile=26/49, Leu=1/49 in human I.		Canonical AA for H2 loop (a4).		Behind H2 loop and may play a direct part in Ag binding (Δ5). Ile not seen in mouse IIB or human I. T=21/49 in human I.		Distal to BS. T=26/50, A=4/50, S not seen in human I.		
gions (Con	Surface or Buried	buried		buried		surface		surface		
Ab Act-1 V _H reg	Act-1 RH V _H (SEQ 1D NO:55)	L	Т	V	D	1	S	₹	S	£-
Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont')	human donor 21/28CL (SEQ ID NO:10)	In	Т	R	D	· ·	S	∢	S	Т
ed in the design	Human I (SEQ ID NO:54)	_	T	A	D	H	*%	T	N	T
id sequences use	Mouse IIB (SEQ ID NO:53)	.	T*	>	D*	×	S	_{\$}	S	.
ent of amino ac	Mouse Act-1 (SEQ ID NO:9)	7	L	^	Q	·	S	S	S	T
Alignme	FR or CDR									
	#	70	71	72	73	74	75	76	77	78
Table 4.	Kabat	69	70	71	72	73	74	75	76	77

	Comment				Q=163/194 in mouse IIB. E=35/50, Q=11/50 in human I.			·		Distal to BS. R=33/51, T=4/51 in human I.			
ions (Cont.	Surface or Buried				half D buried Q C C C C C C C C C C C C C C C C C C					surface			
Ab Act-1 V _H reg	Act-1 RH V _H (SEQ ID NO:55)	А	Y	М	μl	Т	S	S		ଅ	N	E	D
sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont')	human donor 21/28CL (SEQ ID NO:10)	A	Y	M	ជា	Т	S	S	L	~	S	Щ	D
d in the design o	Human I (SEQ ID NO:54)	· ·	X	M	ш	. 1	·	S	*1	x	S	ш	D*
id sequences use	Mouse IIB (SEQ ID NO:53)	4	<u>۲</u> *	M	O	*1	S	S	r.	. *L	*	a	D*
Alignment of amino acid	Mouse Act-1 (SEQ ID NO:9)	V	X	×	0	7	S	S	T	L	S	Ħ	D
Alignme	FR or												
	#	79	08	81	82	83	84	85	98	87	88	68	8
Table 4.	Kabat	78	79	80	81	82	82A	82B	82C	83	84	85	98

Table 4.		Alignme	nt of amino aci	d sequences used	d in the design o	Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont')	Ab Act-1 V _H reg	ions (Con	1t')
Kabat	#	FR or	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ ID NO:55)	Surface or Buried	Comment
87	91		w	*%	Т	Т	Ι	surface	Distal to BS. T=48/51, S=2/51 in human I.
88	92		A	A*	A	A	Y		
68	93		Λ	· *A	Λ	Λ	Λ		
06	94		Ā	*Å	Y*	Y	Y		
91	95		Å	Å	Y	Å	Y		Packing AA. Most common AA.
92	96		2	*0	C*	C	C		
93	62		٧	A	A*	Ą	V		Packing AA. Most common AA.
94	86	FR3	R	R	R	R	R		Canonical AA for H1 loop.
56	66	CDR3	Ð	X	A	g	G		Packing AA. 2 nd most common residue seen at this point - OK.
96	100		G	Y	P	ı	g	·	
26	101		Y	Y	Ŋ	ı	Y		
86	102		D	ß	Y	ı	D		

		ı	1				T	_	Ι.	I	1	1	Г	ı	1	1	
ıt')	Comment						Packing AA. 1=26/1211. F and M are most commonly seen.										Packing AA. Most common AA.
gions (Con	Surface or Buried	·															
LAb Act-1 V _H re	Act-1 RH V _H (SEQ ID NO:55)	9	*	D	¥	A									D	Y	W
Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont')	human donor 21/28CL (SEQ ID NO:10)	g	•		•	•	-	ı	Y	Y	G	S	g	S	Z	γ	W
ed in the design	Human I (SEQ ID NO:54)	D	S	g	9	Ŋ	υ	Ϋ́	R	9	Q	¥	×	뚀	D	Y	W*
id sequences use	Mouse IIB (SEQ ID NO:53)	Ð	S	S	×	×	>	Υ	×	١.	γ*	Ж	γ	F	D	Y	W*
ent of amino ac	Mouse Act-1 (SEQ ID NO:9)	D D	W	D	Y	A	1	1		ı	ı	ı		-	D	Y	W
Alignm	FR or CDR															CDR3	FR4
	#	103	104	105	106	107	108								109	110	111
Table 4.	Kabat	66	001	100A	8001	D001	Q 001	3001	100F	100G	100H	1001	1001	100K	101	102	103

Table 4.	امر	Align	Alignment of amino acid	acid sequences	used in the desig	sequences used in the design of reshaped human mAh Act-1 V regions (Cont')	mAh Act-1 V	regions (C	onth	
Kabat	*	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ ID NO:55)	Surface or Buried	Comment	
104	112		Ð	*5	Ð	Ð	Ö			
105	113		0	0	0	0	0			
106	114		g	*D	*5	9	0			
107	115		Т	*-	⊢	F	Т			
108	911		S	H		1.	1	surface	Distal to BS. T=88/149 in mouse IIB. L=25/39, T=7/39 in human I	2
601	11,7		>	>	*^	>	>		T	.0-
110	118		Ţ	*	L	L	Τ			
111	611		>	*^	>	>	^			
112	120		S	۸*	*%	S	S			
113	121	FR4	S	S	S*	S	S			

Table 3.	#	Alignme FR or	Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _L regions (Cont') FR or Mouse Act-1 Mouse K-II Human K-II GM 607CL Act-1 or RH V _K Surface	equences used in	the design of res	shaped human m	Act-1 V _L regi	ons (Cont	Comment
	£	CDR	(SEQ ID NO:7)	(SEQ ID NO:50)	(SEQ ID NO:51)	(SEQ ID NO:8)	(SEQ ID NO:52)	or Buried	Comment
16	96		D	D	٧	¥	S		Canonical for L3/Packing AA. 3 rd most common AA.
92	97		Т	Т	Г	Г	Т		Canonical AA for L3 loop.
93	86		Н	Н	٥	8	Н		Canonical AA for L3 loop.
94	66		Q	۸	×	Т	ð		Canonical AA for L3 loop.
95	100		Р	p*	Р	Р	P		Canonical AA for L3 loop.
95A			•	Р	R*		-		
95B			•	•	-		-		
95C			•	•	-		-		
95D			•	•			•		
95E			-	•			1		
95F			1	,	ı		-		
96	101		Ϋ́	Y	х	Q	Y		Packing AA. 2 nd most common AA.
97	102	CDR3	Т	T*	Т*	T	Т		Canonical for L3.

nt')	Соттеп	Packing AA. Most common AA.		Distal to BS. Q=12/13, G=1/12 in human k-II.				Distal to BS. L=5/14, V=9/14 in human κ-II.				
gions (Co	Surface or Buried			half buried				half buried				
Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _L regions (Cont')	Act-1 or RH V _K (SEQ ID NO:52)	Ę.	Ŋ	ð	Ð	Τ.	×	>		-		×
reshaped human	GM 607CL (SEQ ID NO:8)	Ŧ.	D	8	Ð	Т	Ж	>	Э	I	-	К
n the design of	Human ĸ-II (SEQ ID NO:51)	F* .	. 9	8	* 9	Т*	Ж	^	Е	*	1	K
sequences used i	Mouse k-II (SEQ ID NO:50)	· * 4	*D	G	*D	Т*	K*	· 1	E*	I	•	K*
nt of amino acid	Mouse Act-1 (SEQ ID NO:7)	ĮŦ.	D	Ö	9	Т	К	ı	Е	1		K
Alignme	FR or CDR	FR4										FR4
	#	103	104	105	106	· 107	108	109	110	111		112
Table 3.	Kabat	86	66	100	101	102	103	104	105	106	106A	107

Replace Table 4 at pages 65-77 with the below Table 4, presented on pages xv-xxvii and marked up by way of bracketing and underlining to show the changes relative to the previous version of the table.

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions.

2 2 2 3 3		(SEQ ID NO:9)	(SEQ ID NO:53)	(SEQ ID NO:54)	(SEQ ID NO:10)	(SEQ ID NO:55)	or Buried	
	FR1	Ø	Ò	Q	0	Q		-
		^	*^	Λ	۸	۸		
		ð	٥*	۷*	Q	8		
4		I	T*	r.	Г	Г		
8		Ø	ð ·	>	> ·	>	surface	Distal to binding site (BS). Q=135/143 in mouse IIB. V=49/53,
								Q=1/53 in human I.
9 9		0	0	0	0	0		
7 7		<u>α</u>	۵	* 2	Ø	ω l	half buried	Distal to BS. P=102/150 in mouse IIB. P not seen in human I.
∞ ∞		. Đ	G*	G *	. D	Ð		
6 6		А	А	٨	Ą	¥		
10 10		E	E	E	E	E		
11 11		Г	1.*	Λ .	^	>1	surface	Distal to BS. V=50/54, L=4/54 in human I.

		re, use e. in I.				/52, nan I.			
	Comment	Pointing into core, but standard mouse to human change. K=41/55, V=3/55 in human I.				Distal to BS. T=12/139, A=117/139 in mouse IIB. T=1/52, A=23/52 in human I.			
(Cont')	Surface or Buried	buried				surface			
Act-1 V _H regions	Act-1 RH V _n (SEQ ID NO:55)	K	К	d	9	₹	S	۸	¥
Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont')	human donor 21/28CL (SEQ ID NO:10)	×	X.	Р	·	A	S	۸	K
in the design of	Human I (SEQ ID NO:54)	x	**	*d	*5	ď	**	. ^	К
sequences used	Mouse IIB (SEQ ID NO:53)	*>	×	*d.	. 0	ď	*\$	^*	К
t of amino acid	Mouse Act-1 (SEQ ID NO:9)	>	ъ.	۵	ŋ	£-	S	>_	×
lignmen	FR or CDR								
Α	#	12	13	14	15	91	17	18	19
Table 4.	Kabat	12	13	14	15	91	17	18	61

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont')

Comment	Pointing into core, but standard mouse to human change. L=138/179 in mouse IIB. V=36/52, L=1/52 in human I.				Canonical AA for H1 loop (A1). G not seen in mouse IIB. G=12/51, A=34/51 in human 1.	Pointing away from BS and so does not appear to bind antigen (Ag). Y=1/185 in mouse IIB. S=48/50, Y not seen in human I.
Surface or Buried	buried				buried	surface
Act-1 RH V _H (SEQ ID NO:55)	>	S		×	U	· νοι
human donor 21/28CL (SEQ ID NO:10)	٨	S	O	К	·	S
Human I (SEQ ID NO:54)		S	*	К	¥	*%
Mouse IIB (SEQ ID NO:53)	L	*S	*2	K*	A*	*
Mouse Act-1 (SEQ ID NO:9)	L	S	C.	К	Ð	, ,
FR or CDR						·
#	20	21	22	23	24	25
Kabat	20	21	22	23	24	25

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Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 $V_{\rm H}$ regions (Cont')

*	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ 1D NO:55)	Surface or Buried	Comment
— -		D	*5	*	5	D		Canonical AA for H1 loop.
		>	>	¥	>	γ		Canonical AA for H1 loop.
		F	È-	£-	F	Т		Canonical AA for H1 loop.
		Įr.	<u>*</u>	#	FI.	F		Canonical AA for H1 loop.
	FR1	Į-		L	Т	Т		Canonical AA for H1 loop.
1	CDR1	S	W	S	S	S		Canonical AA for H1 loop.
1		*	*	¥	γ	Y		Canonical AA for H1 loop.
1		М	. **	¥	Y	w		
		Σ	Σ		M	M		Canonical AA for H1 loop.
35		H	Н	S	Н	н		Packing AA. Most common AA.
1		,	1	1	•			
1	CDRI	•	•	•				
36	FR2	W	W*	*M	W	W		

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont')

		-xix-	
Comment	Packing AA. Most common AA.	Pointing into core, but standard mouse to human change. K=177/188 in mouse IIB. R=48/49, K not seen in human I. However, Lys maybe packing H2 loop, therefore consider changing in second version, in conjunction with A40R, if binding poor.	Packing AA. Most common AA.
Surface or Buried		buricd	
Act-1 RH V _{II} (SEQ ID NO:55)	^	ය	0
human donor 21/28CL (SEQ ID NO:10)	^	ж	Ø
Human I (SEQ ID NO:54)	> .	**	*>
Mouse IIB (SEQ ID NO:53)	>	₩.	0
Mouse Act-1 (SEQ ID NO:9)	>	×	0
FR or			
#	37	3 %	39
Kabat	37	38	39

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 $V_{\rm H}$ regions (Cont')

Comment	Pointing into core, but standard mouse to human change. R=160/177 in mouse IIB. A=37/49, R=0/49 in human I. However, Arg maybe packing H2 loop, therefore consider changing in second version, in conjunction with A38K, if binding poor.				Pointing into core, but standard mouse to human change. G=43/48, R=5/48 in human I.
Surface or Buried	buried				buried
Act-1 RH V _H (SEQ 1D NO:55)	₹	q	Ŋ	8	N N
human donor 21/28CL (SEQ ID NO:10)	A	Р	D.	6	R
Human I (SEQ ID NO:54)	<	Ь	Ð	ð	⁵
Mouse IIB (SEQ ID NO:53)	× .	Δ.	Ð	Ò	Ö
Mouse Act-1 (SEQ ID NO:9)	α.	<u>a</u>	Ö	0	5
FR or					
#	04	41	42	43	4
Kabat	04	41	42	43	44

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont')

		Т								γ		. [
Comment	Packing AA. Most common AA.		Packing AA. Most common AA.	Ile Underneath and supporting H2 loop	Met=41/48, Ile=1/48 in human I.					Canonical AA for H2 loop.			Canonical AA for H2 loop.
Surface or Buried				buried									
Act-1 RH V _H (SEQ ID NO:55)	L	Е	W	1		Ð	Ξ	1	D	Ь			S
human donor 21/28CL (SEQ ID NO:10)	L	В	W	M		G	W		N	А			Ð
Human I (SEQ ID NO:54)	r.*	*3	W*	×		G	W	I	Z	P	Å	1	D
Mouse IIB (SEQ ID NO:53)	L*	* E	*	*1		* 9	R	I	Q	b *	•	1	Z
Mouse Act-1 (SEQ ID NO:9)	J	ш	*	_		D	B	I	D	Ь	1	•	S
FR or CDR						FR2	CDR2						
#	45	46	47	48		\$	20	51	52	53			54
Kabat	45	46	47	. 48		49	50	51	52	52A	52B	52C	53

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont')

Table 4.		Alignn	nent of amino a	cid sequences us	ed in the design	Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V _H regions (Cont')	Ab Act-1 V _H reg	ions (Con	f.
Kabat	#	FR or	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ 1D NO:55)	Surface or Buried	Comment
54	55		п	S	Z	Z	E		Canonical AA for H2 loop.
55	99		S	Ð	G	G	S		Canonical AA for H2 loop.
99	57		z	. 5	D	Z	Z		
57	58		Т	Т	Т	Т	T		·
28	59		Z	Z	Z	K	Z		
59	09		¥	Y	Y	Ā	Y		
09	19		z	z	A	S	Z		
61	62		0	E	ð	Q	Q		
·62	63		×	K*	К	К	K		
63	49		Į.	¥.	Ŧ	Ŧ	ĬΞ		
64	65		×	X	ð	Q	K		
65	99	CDR 2	Ŋ	S	g	Ð	G		
99	<i>L</i> 9	FR3	*	**	æ	&	집	surface	Distal to BS. R=39/49, K not seen in human I.
. 19	89		- V	A*	>	>	>	half buried	Distal to BS. V=45/48, A not seen in human I.
89	69		Т	T.*	Т	Т	Т		

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 $V_{\rm H}$ regions (Cont') Table 4.

Kabat	*	FR or CDR	Mouse Act-1 (SEQ ID NEO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ ID NO:55)	Surface or Buried	Comment
69	70		, 1	*1	I .	Ι	T	buried	Leu Underneath and supporting H2 loop (a3). Ile=26/49, Leu=1/49 in human I.
70	71		T	T*	T	L	T		
71	72		>		A	R	Λ	buried	Canonical AA for H2 loop (△4).
72	73		Q	D*	D	D	Д		
73	74		I	X	Ŧ		I	surface	Behind H2 loop and may play a direct part in Ag binding (\$\alpha\$). Ile not seen in mouse IIB or human I. T=21/49 in human I.
74	75		w	v	*x	S	S		
75	76	<u></u>	ω	*o	L	¥	∀l	surface	Distal to BS. T=26/50, A=4/50, S not seen in human I.
76	77		N	N	S	S	S		
77	78		T	T*	Ι	Т	Т		·

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont') Table 4.

	Mouse Act-1Mouse IIBHuman Ihuman donor 21/28CLAct-1 RH V _H SurfaceComment(SEQ ID NO:9)(SEQ ID NO:53)(SEQ ID NO:55)or Buried	A A A A	. Y Y Y	M M M	Q E E E Distal to BS. buried Q=163/194 in mouse IIB.	E=35/50, Q=11/50 in human I.	Γ $\Gamma *$ Γ Γ Γ Γ Γ	S S S	S S	T T* T	T R R BS. R=33/51, T=4/51 in human I.	ν « « « « « « « « « « « « « « « « « « «	3 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 10 10 10 10 10 10 10 10 10 10 10 11 12 12 13 14 15 16 17 18 19 10 10 10 10 10 10 11 12 12 13 14 15 16 17 18 19 10 10 10 10 10 10 10 11 12 12 12	
rangimon.	FR or N	▼				· .								
I auto 4.	Kabat #	78 79	79 80	80 81	81 82		83	82A 84	82B 85	82C 86	83 87	88	85 89	

Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 $V_{\rm H}$ regions (Cont')

							- T					
Comment	Distal to BS. T=48/51, S=2/51 in human I.				Packing AA. Most common AA.		Packing AA. Most common AA.	Canonical AA for H1 loop.	Packing A.A. 2 nd most common residue seen at this point - OK.			
Surface or Buried	surface											
Act-1 RH V _H (SEQ 1D NO:55)	I	А	۸	Y	¥	2	A	R	9	9	Y	D
human donor 21/28CL (SEQ ID NO:10)	Т	A	V	Y	Y	C	Ą	R	Ð	-	-	,
Human I (SEQ ID NO:54)	Т	А	Λ	Y*	¥	C*	A*	R	Ą	P.	Ð	¥
Mouse IIB (SEQ ID NO:53)	*8	*A	*^	*Å	Å	C *	V	R	X	Ā	Y	g
Mouse Act-1 (SEQ ID NO:9)	S	A	۸	Ā	Y	O	ď		9	Ŋ	Y	D
FR or CDR								FR3	CDR 3			
#	16	92	93	94	95	96	26	86	66	100	101	102
Kabat	87	88	68	06	16	92	93	94	95	96	97	86

· -xxv-

-xxvi0

Packing AA. 1=26/1211. F and M are most commonly seen. Packing AA.
Most common
AA. Comment Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V_H regions (Cont') Surface or Buried Act-1 RH V_H (SEQ ID NO:55) ≽ \succ ≥ G \succ ⋖ human donor 21/28CL (SEQ ID NO:10) ≥ \succ G Ö S \mathbf{z} \succ S Ö Human I (SEQ ID NO:54) * * Ö \succ × Ω \succ Ω щ G G Ö G \circ \succ \approx S Mouse IIB (SEQ ID NO:53) ** * ≽ Ω \succ ı Ö × × > \succ × S S Mouse Act-1 (SEQ ID NO:9) ≽ О Ö ≽ Ω \succ ٨ CDR 3 FR or CDR FR4 111 110 108 109 105 901 104 107 103 # Table 4. Kabat 100D 100G 100H 100K 100E 100A 100B 100C 100F 100J 100I 103 101 102 100 66

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Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 $V_{\rm H}$ regions (Cont') Table 4.

		,							
Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V _H (SEQ ID NO:55)	Surface or Buried	Comment
104	112		Ü	·9	Ð	D	G		
105	113		ð	0	Q.	6	8		
106	114		. 9	*5	· 0*	9	Ö		
107	115		T	T*	Т	Т	Т		·
108	116		w		1	L	ıl.	surface	Distal to BS. T=88/149 in mouse IIB. L=25/39,
									T=7/39 in human I.
109	117		>	Λ	^*	Λ	>	_	
110	118		T	Т*	Т	т.	Т		
Ξ	119		>_	*^	۸	Λ	>		
112	120		S	% *	S*	S	N		
113	121	FR4	S	S	, *S	S	S		